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Anthrax vaccine: a review

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Anthrax is a zoonotic disease, primarily of ruminants, that is caused by *Bacillus anthracis*. The three most common forms of anthrax are cutaneous, inhalational, and gastrointestinal. In the 1870s, Robert Koch cultured *B anthracis* and first established the microbial origin of an infectious disease. In 1881, Pasteur and Greenfield independently attenuated *B anthracis* and developed successful vaccines for livestock [1]. In the late 1800s and early 1900s, rag pickers in Germany and wool sorters in England developed cutaneous and inhalational anthrax [2].

Because of livestock immunization programs, natural anthrax is not a major public health problem in the United States, although it is in other parts of the world. Natural cases principally are associated with unusual agricultural, industrial, or laboratory exposure.

Agricultural cases occur primarily in Asia and Africa. Industrial cases occurred primarily in Europe and North America. In the United States, most human cases initially were reported in industrialized northeast states. The location of human cases shifted as the textile industry moved to other parts of the country.

An unusual epidemic occurred in Sverdlovsk, Russia, in 1979. After the accidental release of spores into the atmosphere from a military microbiology facility, at least 77 human cases of inhalational anthrax occurred, leading to at least 66 deaths [3,4]. Iraq's 1995 admission to the United Nations that it produced weapons containing anthrax spores confirmed the potential use of *B anthracis* as a biologic weapon [5]. The United States and the United Kingdom had sizable and advanced biologic-weapons programs until the 1970s.

Growing recognition of the threat of *B anthracis* as a biological weapon led the US Department of Defense to begin anthrax vaccinations for selected members of the Armed Forces in March 1998. The intentional use of anthrax spores as a weapon in fall 2001 along the US eastern seaboard substantially altered public perceptions of anthrax. In late September 2001, a Florida man developed a fatal

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case of inhalational anthrax, the first US case since 1976 [6]. He was the first of five deaths among 11 confirmed inhalational cases and seven confirmed and four suspected cutaneous cases of anthrax. Exposure to contaminated mail was the confirmed or apparent source of infection in all patients [7].

Background

Clinical description

Anthrax occurs in three primary forms: cutaneous, inhalational, and gastrointestinal [8,9]. Secondary meningitis can occur with all three forms. In the United States, about 95% of reported cases have been cutaneous, and 5% have been inhalational. No confirmed case of gastrointestinal anthrax has been identified in the United States.

The incubation period for cutaneous anthrax ranges from 1 to 7 days [8,9]. Cutaneous anthrax first is noted as a small, pruritic papule. During the following several days, the papule develops into a vesicle that is 1 to 3 cm in diameter and may become hemorrhagic. Systemic symptoms include mild malaise and low-grade fever, with regional lymphangitis and lymphadenopathy. From 5 to 7 days after manifestation, the vesicle ruptures, revealing a straight-edged, depressed crater that develops a typical black eschar. After 2 to 3 weeks, the eschar falls off, usually without leaving a scar. Mortality in untreated cutaneous cases is about 20%, but less than 1% with antibiotic therapy. The cause of death in cutaneous anthrax is the eventual dissemination of the bacillus in the absence of treatment.

With inhalational anthrax, nonspecific symptoms develop 1 to 5 days after inhaling an infectious dose of *B anthracis* organisms [8,9]. These symptoms include malaise, fatigue, myalgia, slight temperature elevation, and minimal nonproductive cough. Within 2 to 4 days, a slight improvement may occur. Then, severe respiratory distress, including dyspnea, cyanosis, and profuse diaphoresis, develops suddenly. Widening of the mediastinum on chest radiograph and pleural effusions are common. Shock may develop, with death usually following within 24 hours. Death probably is caused by lymphatic or vascular obstruction in the mediastinum with pulmonary hemorrhage and edema. Inhalational anthrax is almost always fatal if left untreated.

Among the inhalational cases treated in 2001, none initially had a normal chest radiograph [10]. Six of the 11 (55%) patients survived with aggressive clinical support and multiple antibiotic therapy [10,11]. All four individuals who initially exhibited fulminant signs of illness with severe respiratory distress or hypotension or meningitis died, despite receiving antibiotics active against *B anthracis*.

Gastrointestinal anthrax symptoms develop 2 to 5 days after ingesting contaminated meat [8,9]. The initial symptoms of disease include nausea, vomiting, anorexia, and fever. Physical examination reveals an elevated temperature, pulse, and respiratory rate. Sepsis with toxemia, shock, and death may develop. Gastrointestinal cases have an untreated mortality rate of 25% to 75%.

Bacteriology

B anthracis is a large, Gram-positive, spore-forming, nonmotile bacillus. It readily grows aerobically on sheep blood agar and without hemolysis. In high concentrations of carbon dioxide, the organisms form antiphagocytic capsules. In tissue, the bacteria are encapsulated, appearing singly or in chains of two or three bacilli. Anthrax bacteria are identified by the production of toxin antigen, lysis by a specific gamma bacteriophage, presence of capsule and cell-wall polysaccharide (recognized by fluorescent antibody), and virulence for mice and guinea pigs. Polymerase chain reaction (PCR) tests for toxin and capsule genes are also confirmatory. Based on genetic analyses of multiple isolates, *B anthracis* is one of the most monomorphic, homogeneous bacterial species [12,13].

Anthrax spores resist environmental extremes, surviving for decades in certain soil conditions. Studies have shown that viable spores can persist for weeks to months within the lungs of Rhesus monkeys after inhalation, at which time they were still capable of germinating and causing fatal infection [14–17].

Pathogenesis

For anthrax, the known virulence determinants that are important in pathogenesis are the bacterial capsule and two protein exotoxins. Bail demonstrated that organisms that lost the ability to produce capsule were avirulent [18]. In the 1930s, Sterne and colleagues, showed that such nonencapsulated strains could induce immunity to anthrax [19], showing that a capsule is not necessary to elicit protective immunity.

Genes encoding the anthrax capsule are carried on an extrachromosomal 96-kilobase (kb) plasmid (pX02) [20,21]. Anthrax strains without the capsule plasmid do not produce a capsule and are attenuated [22]. The capsule makes the organism resistant to phagocytosis and may prevent lysis by cationic proteins in serum [23]. Although the capsule is necessary for virulence, it is not an effective immunogen in most experimental animals.

Smith and Keppie established the role for toxins in anthrax pathogenesis by demonstrating that sterile plasma from experimentally infected guinea pigs was lethal when injected into other animals [24]. *B anthracis* has been described as a large bacterium that produces a feeble toxin [25]. The two anthrax toxins possess a binding domain to attach to target-cell receptors and an active domain for biochemical activity. The toxins share the same binding protein, called protective antigen (PA). PA, combined with a second protein called lethal factor (LF), constitutes the anthrax lethal toxin, so-named because it is lethal when injected into experimental animals [26,27]. PA combined with a third protein, edema factor (EF), constitutes the edema toxin, which causes edema in experimental animals. The edema toxin causes massive edema, especially inhalational anthrax. Each of the three proteins alone lacks biologic activity.

The crystalline structures of PA, LF, and EF are known [28–30]. The current model suggests that PA first binds to an anthrax toxin receptor [31]. PA is cleaved

by a cell-surface protease, releasing a 20-kd amino-terminal fragment. The cell-bound, 63-kd, carboxy-terminal fragment heptamerizes and creates a second binding domain to which LF or EF binds. The complex enters the cell through endocytosis and exerts its toxic effect within the cytosol.

The genes for the toxin proteins are carried on a second 182-kb plasmid (pX01) [32]. The pathogenic role of these toxins was shown by the attenuation of strains that are devoid of the plasmid coding for the toxin genes but still are encapsulated [22,32]. Deleting the PA gene alone eliminates the organism's virulence [33], establishing the pivotal role of PA in toxin activity and virulence.

Infection begins when spores are introduced through the skin or mucosa. A spore germinates into the vegetative bacillus, producing the antiphagocytic capsule. The edema and lethal toxins produced by the organism degrade leukocyte function and contribute to tissue necrosis, edema, and relative lack of leukocytes. If not contained, the bacilli spread to the draining regional lymph node, leading to further toxin production and induction of hemorrhagic, edematous, and necrotic lymphadenitis. From lymph nodes, the bacteria multiply and enter the bloodstream to produce a systemic infection.

In inhalational anthrax, spores are ingested by alveolar macrophages and are transported to tracheobronchial and mediastinal lymph nodes, where they germinate [34]. The bacilli spread through the blood, causing septicemia. Late in disease development, toxin is present at high concentrations in the blood [35]. The role of lethal toxin in producing death remains obscure, but may involve uncontrolled release of cytokines and other possible mediators from macrophages. Death results from respiratory failure with overwhelming bacteremia, which often is associated with meningitis and subarachnoid hemorrhage.

Diagnosis

A diagnosis of cutaneous anthrax should be considered after a painless, pruritic papule appears, develops into a vesicle, and reveals a black eschar. The diagnosis should be confirmed by Gram's stain or culture of vesicular fluid. Previous antibiotic treatment quickly renders the infected site as having negative culture results. A biopsy specimen that is taken at the lesion edge and examined using Gram's stain, immunohistology, and PCR tests may be useful after beginning antibiotic therapy [8,9].

The diagnosis of inhalational anthrax is difficult, except in cases with a history of exposure to an aerosol with *B anthracis*. In its early stage, the symptoms of inhalational anthrax are nonspecific. Once the acute stage develops, a widened mediastinum can be seen on a chest radiograph, often with pleural effusions, and suggests the diagnosis. In untreated cases, cultures of blood and pleural effusions readily establish the diagnosis. In cases previously treated with antibiotics, PCR tests of blood and pleural fluid and immunohistochemical examination of pleural fluid or transbronchial biopsy specimens may help [10,11,36]. Because primary pneumonia is not a usual feature, sputum examinations do not aid diagnosis. In the radiographic differential diagnosis, histoplasmosis, sarcoidosis, tuberculosis,

and lymphoma should be considered. CT of the chest may help detect mediastinal hemorrhagic lymphadenopathy and edema, peribronchial thickening, and pleural effusions.

Gastrointestinal anthrax is difficult to diagnose because of its rarity and similarity to other, more common severe gastrointestinal diseases [8,9]. An epidemiologic history of ingesting contaminated meat suggests the diagnosis. Microbiologic cultures do not help confirm the diagnosis, unless bacteremia is present.

Treatment and prevention with antibiotics

Mild cases of cutaneous anthrax effectively may be treated orally with a penicillin, a tetracycline, or another antibiotic, until specific antimicrobial resistance is known. If spreading infection or prominent systemic symptoms are present, high-dose parenteral therapy should be considered (as in inhalational anthrax), until a clinical response develops. Effective therapy reduces edema and systemic symptoms but does not change the evolution of the skin lesion itself.

Treating inhalational or gastrointestinal anthrax requires high-dose intravenous therapy with two or more antibiotics, including a fluoroquinolone or doxycycline [7,10,11,17,37–40]. Limited animal data suggest that adding an aminoglycoside to penicillin treatment may provide additional benefit. Regimens should be altered based on susceptibility testing and clinical status. Successful treatment of 6 of the 11 inhalational cases in 2001 suggests that rapid treatment and modern supportive care can lower mortality expectations to those of other causes of sepsis.

Prophylactic treatment to prevent anthrax after exposure to an infectious spore aerosol involves the use of oral antibiotics for at least 30 to 60 days, depending on individual circumstances (eg, extent of exposure, vaccination status) [40–43]. The Food and Drug Administration affirmed evidence for the safety and efficacy of ciprofloxacin, doxycycline, and penicillin G procaine for this indication [39]; amoxicillin is recommended for children and pregnant or lactating women, depending on microbial sensitivity [11,37,38,40,44]. Pre- or postexposure vaccination may enable shorter courses of antibiotics [40,41,45]. Postexposure vaccination alone is not expected to be protective [17].

Epidemiology

Several theories address the ecology of soil infected with *B anthracis*. According to one theory, *B anthracis* spores persist in soil for many years under certain conditions [46]. Survival is favored in soil that is rich in nitrogen and organic material and has adequate calcium, a pH greater than 6.0, and an ambient temperature greater than 15.5°C. It remains unclear whether cycles of germination occur within the soil or whether amplification within mammals maintains the spores in the soil between animal outbreaks.

Animal anthrax results from ingesting *B anthracis* spores while eating contaminated feed or grazing on pastures [46]. Soil becomes contaminated from contaminated fertilizer or contaminated feed spread on the ground or from diseased animals that contaminate the soil with their secretions before or after death.

Reported human anthrax cases in the United States declined steadily in the 20th century. The annual average number of cases between 1916 and 1925 was 127; between 1948 and 1957 was 44; between 1978 and 1987 was 0.9; and between 1988 and 2000 was 0.25 [47,48]. The anthrax terror attacks of fall 2001 resulted in 11 confirmed inhalational cases and 7 confirmed and 4 suspected cutaneous cases [7,10,11]. More than 32,000 people received prophylactic antibiotics while potential exposures were evaluated [42].

Passive immunization

Before the antibiotic era, antisera harvested from animals were common therapeutic products [49]. One of the first of such products was anthrax antiserum, which was developed in France by Marchoux and in Italy by Sclavo in 1895 [50,51]. Initially used for prophylaxis and treatment of anthrax among livestock, Sclavo later used his product to treat human disease. He reported 10 deaths among 164 treated patients (6% mortality rate, compared with a normal rate of 24%).

From the 1910s to 1940s, clinicians in Europe and the Americas treated patients with anthrax antiserum using 25 to 300 mL daily for 5 days, sometimes in combination with arsenicals [49–54]. No controlled studies were performed to demonstrate efficacy. Sulfanilamide superceded anthrax antiserum, followed by penicillin and other antibiotics. [55,56]

Passive immunization with equine antibody that was produced against attenuated Sterne veterinary vaccine strains or against crude toxins prevented disease in animals when given before or shortly after spore challenge [14,57]. One or two doses of hyperimmune serum containing equine anti-anthrax spores protected Rhesus monkeys when given 1 day after low-dose aerosol challenge. Forty-five percent of serum-treated animals survived, compared with 10% of controls.

Little and colleagues showed that anti-PA antiserum protected against intramuscular challenge in animals [58]. The anti-PA polyclonal antibody protected against death, and anti-PA monoclonal antibody significantly delayed mortality. Reuveny and colleagues similarly found that passive immunization of guinea pigs with polyclonal anti-PA antisera conferred protection against intradermal challenge [59].

Kobilier et al challenged guinea pigs intranasally with spores and then treated the animal with anti-PA, anti-LF, or anti-Sterne vaccine antibodies [60]. Intraperitoneal administration of rabbit anti-PA serum 24 hours after infection protected 90% of infected animals; anti-Sterne and anti-LF antibodies had lesser efficacy.

Beedham et al protected mice against challenge with a vaccine strain using serum, but not spleen lymphocytes, from PA-vaccinated animals. These findings

support the conclusion that antibody is the major mediator of vaccine-induced immunity [61].

Even though the contributions of anthrax toxins to pathogenesis suggests that antisera may be beneficial, Western interest in such products did not arise until the anthrax bioterrorist attacks of fall 2001 [62]. The need for therapeutic options other than antibiotics would be enhanced in treating antibiotic-resistant strains of *B anthracis*.

Active immunization

Unlike live attenuated veterinary vaccines, most modern human anthrax vaccines consist of proteins purified from anthrax cultures. Early human anthrax vaccines were developed in the 1910s, but found little favor [51]. Sterne developed his live, attenuated strains in the 1930s, which still are used worldwide for domesticated animals [19,49,63]. Russian investigators developed vaccines similar to Sterne's for animal and human use. In 1946, Gladstone identified the PA component of *B anthracis* [64]. Belton and Strange increased PA yield to allow large-scale production [57], leading to the current British vaccine. Wright and colleagues used similar techniques to develop precursors to the current American vaccine [65,67].

The major effective immunogen in culture supernatants is PA. Although smaller amounts of LF and EF may be present; their contribution to protective immunity is unclear [68]. In older studies, EF enhanced the protective efficacy of PA in some experimental animals [69,70]. The results of these studies are difficult to interpret, because the preparations may not have been pure. Studies using the PA gene cloned into *B subtilis* demonstrated that PA alone, without LF, EF, or other proteins, protects animals against experimental infection [71]. Other experiments showed that pure PA (free of detectable LF or EF) [72] or recombinant PA [73] can protect experimental animals. It remains unknown whether adding LF or EF enhances the protective efficacy of PA.

The human anthrax vaccine licensed in the United States since 1970, anthrax vaccine adsorbed (AVA), is produced by the BioPort Corporation from sterile filtrates of microaerophilic cultures of an attenuated, unencapsulated nonproteolytic strain (V770-NP1-R) of *B anthracis*. The cell-free culture filtrate, containing principally PA, is adsorbed to aluminum hydroxide. Benzethonium chloride 0.0025% is added as a preservative, and formaldehyde not exceeding 0.02% is added to stabilize the preservative. The manufacturer adopted the trade name *BioThrax* in early 2002. BioPort's current standards require 5 to 20 μg/mL of total protein, of which at least 35% is the 83-kd PA protein [74]. Potency testing of the BioPort vaccine is performed by assessing biologic activity after parenteral challenge in guinea pigs.

Some lots produced in Lansing in the 1980s seemed to contain small amounts of LF and lesser amounts of EF, as determined by induction of antibody responses in animal recipients [22,72,75,76]. This effect has not been reported

in limited observations in human vaccinees [76]. One analysis found no detectable EF by Western blotting. ELISA studies found LF to be present in the range of 10 to 30 ng/mL of fermentation filtrate before adsorption [74]. A mouse macrophage cytotoxicity assay has suggested that LF is present in a biologically inactive form.

The US-licensed vaccine is stored at 2°C to 8°C. The recommended schedule for vaccination is 0.5 mL given subcutaneously at 0, 2, and 4 weeks, followed by 0.5-mL boosters at 6, 12, and 18 months [74]. With continued risk for exposure, additional yearly boosters are recommended. Preliminary studies suggest that the vaccine is as immunogenic and less reactogenic when administered intramuscularly and at prolonged dosing intervals [77–80]. Expanded studies are underway of immunogenicity with intramuscular administration and fewer doses.

Anthrax vaccine precipitated, a similar vaccine from the Centre for Applied Microbiological Research (Porton Down, Salisbury, United Kingdom), first was administered to humans in the early 1950s and licensed in the United Kingdom in 1979 [1,81–84]. This vaccine is made by precipitating the sterile cell-free culture filtrate of a derivative of the attenuated, noncapsulating Sterne strain $34F_2$ with aluminum potassium sulfate [83]. LF and EF are present at levels higher than those in lots of the US vaccine from the 1980s [76,85]. The vaccine contains thimerosal as a preservative. The British vaccine is administered intramuscularly in a regimen of three 0.5-mL doses at 0, 3, and 6 weeks, with a booster dose 6 months after the third dose. Subsequent booster doses are given annually as long as the exposure risk persists [1].

A human vaccine consisting of a suspension of live spores, named STI-1, has been manufactured in the former Soviet Union and its subsequent independent republics since 1953 [46,86]. This strain, similar to the Sterne strain used in veterinary vaccines, is unencapsulated [86]. Although the STI-1 vaccine has a reputation for inducing substantial side effects, its developers assert that it is reasonably well tolerated and shows some degree of protective efficacy [86–88]. Manufactured by the Tblisi Scientific Research Institute of Vaccines & Serums (Tblisi, Georgia) and the Institute of Microbiology (Kirov, Russian Federation), this vaccine is given by subcutaneously or scarification through a 10- to 20-μL drop of vaccine containing 1.3 to 4.0×10^8 spores [1,46,84,86,88]. A second dose follows 21 days later, with yearly boosters.

Another live-spore human vaccine given by scarification has been manufactured by the Lanzhou Institute of Biological Products (People's Republic of China) since the 1960s and is based on avirulent strain A16R [46,89]. A single dose contains 1.6 to 2.4×10^8 colony-forming units. A single booster dose is given 6 to 12 months after the first vaccination.

Immunogenicity and efficacy

In one study, the US-licensed vaccine induced an immune response to PA (measured by indirect hemagglutination) in 83% of vaccinees 2 weeks after the first three doses [90]; in another study, tin induced a response in 91% of vaccinees after

receiving two or more doses [91]. Titer levels decreased over time, but 100% of vaccinees responded with an anamnestic response to the annual booster dose. An analysis using a more sensitive anti-PA ELISA demonstrated that seroconversion occurs in 96% to 100% of vaccinees after the second dose [77].

Using a refined, validated ELISA assay, Pittman et al found that one dose of AVA evoked detectable anti-PA IgG antibodies in 60% to 84% of vaccines [78]. After two doses, 95% to 100% of vaccinees developed anti-PA antibodies. Prolonging the interval between the first two doses by a few weeks beyond the licensed 2-week interval increased antibody response [79]. More extended intervals did not impair booster responses among Gulf War veterans who were given anthrax and botulinum vaccines after gaps of 18 to 24 months [80].

After a naturally acquired infection, antibody to PA has been shown to develop in 68% to 93% of cases, depending on the time when samples were drawn [85,91–93]. Antibody to LF occurs in 42% to 55% of cases, whereas antibody to EF is observed less frequently [85,92]. Antibody to the anthrax capsule occurs in 67% to 94% of cases [92,93]. In the 2001 anthrax outbreak, all survivors developed anti-PA antibodies [7].

In experimental animals, there is generally a correlation between immunity and antibody titer to PA after immunization with the human vaccine [94]; however, the live veterinary vaccine provides significantly greater protection against anthrax in experimental animals than does the human vaccine, even though it often induces lower levels of antibody to PA [72,75,76], suggesting that other antigens may be involved in protection.

More recent studies have shown a strong correlation between antibodies to PA and immunity. Using live vaccines that produced varying amounts of PA, Barnard and Friedlander showed that protection strongly correlates with antibody titers to PA [95], a finding that was confirmed by Cohen and colleagues [96]. In a rabbit model of inhalational anthrax using the US-licensed human vaccine, Pitt et al found a similar in vitro correlation of immunity with antibody to PA, measured by ELISA and toxin neutralization [94]. Using a PA vaccine to protect guinea pigs against an intradermal challenge, Reuveny et al found that toxin-neutralizing antibodies correlated better with survival than did antibodies measured by ELISA [59]. The protective efficacy of experimental PA-based vaccines that are derived from culture filtrates of *B anthracis* was demonstrated with the use of various animal models and routes of challenge [68,83].

The Centers for Disease Control and Prevention sponsored a controlled human field with a less-potent vaccine similar to the current US-licensed vaccine [66,74,97]. The study was conducted among vulnerable workers at four textile mills in the northeastern United States, where raw imported goat hair contaminated with *B anthracis* was processed. The results indicated that vaccination, compared with placebo treatment, provided 92.5% protection against anthrax, combining the cutaneous and inhalational cases (95% confidence interval, 65%–100%). No isolated assessment of the effectiveness of the vaccine against inhalational anthrax could be made, because there were too few inhalational cases. Inhalational cases occurred only among unvaccinated workers and not among vaccinated workers.

A Cochrane review of this human field trial found the less-potent vaccine to be effective; a trial with the live spore vaccine developed in the former Soviet Union also found its tested vaccine to be effective [88]. The current US-licensed anthrax vaccine protected 62 of 65 vaccinated Rhesus monkeys against an aerosol exposure of anthrax spores, in contrast to no survivors among 18 unvaccinated monkeys [17,73,74,88,98–101]. Considering all extant studies, a peer-reviewed evaluation by the National Academy of Sciences reported that "The committee finds that the available evidence from studies with humans and animals, coupled with reasonable assumptions of analogy, shows that AVA as licensed is an effective vaccine for the protection of humans against anthrax, including inhalational anthrax, caused by all known or plausible engineered strains of *B. anthracis*" [74].

Postexposure vaccination by itself is unlikely to be of any benefit because of the short incubation period and the rapid course of the disease [17]. Vaccination combined with antibiotic prophylaxis before clinical illness may offer the best protection against inhalational disease after an aerosol exposure [41]. This outcome is likely because of the unusual propensity of anthrax spores to persist in the host for long periods and possibly germinate after antibiotics have been discontinued [14–17]. Vaccination elicits an immune response during the period of antibiotic prophylaxis. Postexposure vaccination may shorten the period of antibiotic prophylaxis required for protection [41].

Vaccine safety

The US-licensed aluminum hydroxide-adsorbed protective-antigen vaccine, when first used, resulted in an incidence of local reactions similar to that of the alum-precipitated vaccine [67]. In an open-label study from 1966 to 1971 with the US-licensed vaccine, 7000 textile employees, laboratory workers, and others received 15,907 doses [102,103]. There were 24 reports (0.15% of doses) of severe injection-site reactions. There were 150 reports (0.9%) of moderate local reactions and 1373 reports (8.6%) of mild local reactions.

The US Army Medical Research Institute of Infectious Diseases (USAMRIID) assessed the safety of the US-licensed anthrax vaccine between 1996 and 1999 [77]. Each of the 28 volunteers was observed for 30 minutes after subcutaneous administration of AVA and scheduled for follow-up evaluations at 1 to 3 days, 1 week, and 1 month after vaccination. The most common local reactions were tenderness, erythema, subcutaneous nodule, induration, warmth, local pruritus, limited arm motion, and edema. Injection-site reactions occurred more often in women than in men. No abscess or necrosis was observed at the injection site. Systemic reactions included malaise, headache, myalgia, fever, anorexia, respiratory difficulty, and nausea or vomiting. All local and systemic adverse events were transient.

USAMRIID also analyzed the occupational health records of 1583 workers (1249 men) who reported adverse events after receiving 10,722 doses of the US-licensed anthrax vaccine from 32 vaccine lots [104]. Of this group, 273 people received 10 or more doses, and 46 people received 20 or more doses. For injection-

site reactions, 3.6% of doses were reported to produce redness, induration, itching, or edema. Most people who reported a reaction received subsequent doses without problems. Subjects who reported an injection-site reaction were more likely to report a local reaction to a later dose. Systemic events of headache, fever, chills, malaise, and muscle or joint aches were reported after 1% of doses. The most common of these effects were headache (0.4%), malaise (0.4%), and fever (0.1%). Women noted local (ie, erythema, induration, edema, swollen lymph nodes, lumps) and systemic events (ie, headache, fever, dizziness, hives) more commonly than did men. Vaccine recipients younger than 40 years reported adverse events more often than did those 40 years or older.

Two uncontrolled case series used self-administered surveys to assess anthrax vaccine safety. Among healthcare workers at an Army hospital in Honolulu and at a US Army base in South Korea [74,105], women reported more localized itching, subcutaneous nodules, injection-site erythema, fever, and swelling of the lower arm than did men. Regardless of gender, almost all reported events were localized or minor, were self-limited, and did not lead to impairment of work performance.

The most comprehensive evidence evaluating the overall safety of the US-licensed vaccine comes from database studies from the Army Medical Surveillance Activity and the Naval Health Research Center [74,106,107]. These studies established that anthrax-vaccine recipients and nonrecipients of either gender are hospitalized and visit outpatient clinics for the same diseases at the same incidence rates.

All reports to the Vaccine Adverse Event Reporting System (VAERS) involving the US-licensed anthrax vaccine were evaluated by the Anthrax Vaccine Expert Committee (AVEC), comprised of independent civilian physicians [108]. The AVEC evaluated 1857 VAERS reports and additional medical records corresponding to 1793 recipients of the licensed anthrax vaccine between March 1998 and February 2002. The 1857 adverse event reports can be grouped into three main categories based on effect on the vaccine recipient's functional status: hospitalization, inability to work at least 24 hours, and other. Sixty-four of the 1857 reports involved hospitalization. The civilian panel found that 11 of the 64 cases "very likely/certainly" or "probably" were caused by anthrax vaccine. These 11 cases involved allergic or inflammatory reactions at the injection site. Another 172 reports involved the inability to work at least 24 hours (but did not involve hospitalization); 94 of these reports certainly or probably were caused by anthrax vaccine. These 94 reports primarily described injection-site reactions, various rashes, acute allergic reactions, and viral-like symptoms. A total of 1621 reports involved neither hospitalization nor time off work 24 hours or more. All of these cases were reviewed by the AVEC, which found no patterns of unexpected adverse events.

A cohort study involving 4092 active-duty women in the US Army assessed the effect of the US-licensed anthrax vaccine on pregnancy and childbirth [109]. In this cohort, 3135 women who were vaccinated against anthrax were compared with 957 unvaccinated women. There were 39,549 person-months of follow-up. The anthrax-vaccinated and unvaccinated women had an equivalent likelihoods

of becoming pregnant and giving birth. The study found no differences in birth outcomes between the two groups, but the study did not have adequate statistical power to rule out a small effect of vaccination on adverse birth outcome, given the low number of adverse outcomes.

These and other safety studies of anthrax vaccine, some still in the peer-review process before publication of this article, were reviewed critically by the expert committee convened by the National Academy of Sciences [74]. The peer-reviewed report by the National Academy of Sciences concluded that the US-licensed anthrax vaccine has a side-effect profile similar to that of other adult vaccines. According to the reviewers [74]:

> The committee found no evidence that people face an increased risk of experiencing life-threatening or permanently disabling adverse events immediately after receiving AVA, when compared with the general population. Nor did it find any convincing evidence that people face elevated risk of developing adverse health effects over the longer term, although data are limited in this regard (as they are for all vaccines).

Indications

Veterinarians and agricultural workers who have contact with potentially infected animals should be immunized, as should laboratory workers who work with *B anthracis* [41,45,110]. Routine anthrax immunization is warranted for industrial workers who handle potentially contaminated animal products, such as wool, goat hair, hides, and bones imported from countries in which animal anthrax continues to occur. These countries are primarily in Asia and Africa, but occasionally include South America or the Caribbean.

Special circumstances that warrant vaccination with anthrax vaccine include a threat of biologic warfare or terrorism. The US Armed Forces began vaccinating selected service members in 1998 for individual and collective protection against anthrax exposure by way of biologic weapons [74].

A significant hypersensitivity reaction to a previous dose of anthrax vaccine is a relative contraindication to further doses. If it is necessary to immunize such individuals, pretreatment with antihistamines and nonsteroidal anti-inflammatory drugs may be of value, although this approach has not been evaluated scientifically [41,45].

Future efforts

Research is underway to change the current US-licensed vaccine's route of administration to intramuscular and to reduce the number of doses in the basic series. So-called next-generation anthrax vaccines may be composed of PA alone or as a complex with LF or EF. Other research efforts may evaluate adjuvants

other than aluminum [111,112] or new formulations using microcapsules [113]. The most advanced vaccine candidates, based on recombinant PA, protected Rhesus monkeys from inhalational challenge [71,73,114,115], and human clinical trials of these vaccines are underway.

Summary

Anthrax can be a deadly disease if treatment does not begin early in the course of infection. An effective vaccine has been available in the United States since 1970, although it was not used widely until 1998. A comprehensive, peer-reviewed evaluation by the National Academy of Sciences affirmed the findings of multiple previous independent panels that found that the US-licensed anthrax vaccine is safe and effective [40,41,45,74,88,102,108].

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